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measures prevented the misidentification of contaminating, undifferentiated myoblasts which quite likely contributed to the confusion regarding the activity of microtubule depolymerizing agents in myogenic dedifferentiation.

More recent studies have identified other agents, which seem more certain to induce dedifferentiation in vitro. "Reversine" is a small molecule that can trigger myoblasts, which are lineage committed, to dedifferentiate into more multipotent progenitor-type cells which are capable of being directed to differentiate not only into osteoblasts but adipocytes as well [15]. Such discoveries have not been limited to small molecules: Chen et al. recently showed that ciliary neurotrophic factor (CNTF) can similarly induce myoblasts to adopt a multipotent phenotype capable of redifferentiating into adipocytes, glial, and neuronal cells [16]. It remains to be determined whether reversine and CNTF share a common mechanism or have an effect on the more differentiated myotube. Perhaps a combination of a microtubule depolymerizer and reversine might accomplish that which the former, and agents like it, alone cannot—complete cellularization and dedifferentiation of mammalian myotubes.

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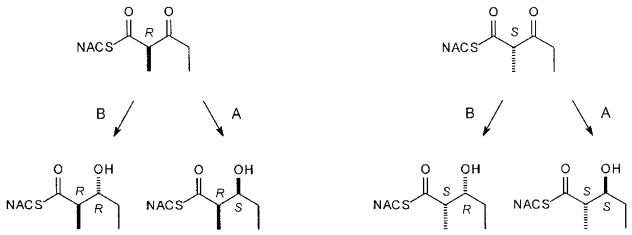
The Stereochemistry of Ketoreduction

In this issue of *Chemistry & Biology*, Leadlay and coworkers [1] report overproduction of a number of ketoreductase domains from modular polyketide synthases. These discrete enzymes allow the stereochemistry of polyketide ketoreduction to be studied in isolation.

The well-studied 6-deoxyerythronolide B synthase (DEBS) catalyzes formation of the macrolactone core of erythromycin [2]. DEBS contains a module for each of the six cycles in the chain assembly process. This modular organization is found in many other important polyketide synthases (PKSs) [3]. In a typical module, an acyl transferase (AT) loads an extender unit onto the phosphopantetheine thiol of an acyl carrier protein (ACP). The extender condenses with an acyl chain that is thioester-linked to the active site cysteine of a ketosynthase (KS). The resulting β-ketoacyl-ACP may be reduced to a β-hydroxyacyl intermediate by a ketoreductase (KR) and may be processed further by dehydratase and enoyl reductase enzymes. An entire PKS consists of a series of modules that are housed within large multienzyme polypeptides. A frequently occurring module catalyzes the incorporation of propionate, and reduction of the β-ketone group to an alcohol. The 2-methyl-3-hydroxyacyl thioester product has two new chiral centers. All four combinations of methyl and alcohol stereochemistry [(2*R*, 3*S*), (2*S*, 3*R*), (2*R*, 3*R*), (2*S*, 3*S*)] can appear in nascent polyketide chains. It is unclear how these different stereochemical outcomes are achieved by PKS modules of apparently similar domain composition and sequence.

Detailed studies on DEBS and truncated derivatives have given deep insights into the stereochemistry of polyketide chain extension. All six modules use (2*S*)-methylmalonyl-CoA as a source of activated propionyl extender units [4]. In the cycle catalyzed by DEBS module 2, condensation proceeds with inversion of stereochemistry, so that the initial product is (2*R*)-2-methyl-3-ketoacyl-ACP2 [5]. With DEBS module 1, the



B - type				
TYLS KR1	**99**	0	1	0
DEBS KR1	0	0	**100**	0
A - type				
DEBS KR2	0	**6**	20	74
DEBS KR5	0	**24**	2	74
DEBS KR6	0	**25**	5	70

Figure 1. Reduction of Diketide Substrates by Isolated KR Domains In Vitro

The yields of each hydroxy diketide are expressed as the percentage of the total products reduced by each KR. Numbers in bold type indicate the product expected for each KR, based on the inferred reaction in the native PKS. The B-type TYLS KR1 and DEBS KR1 selected their natural substrates from the racemic mixture and catalyzed ketoreduction strictly in the normal sense. The A-type DEBS KR2, KR5, and KR6 domains reduced their natural (2*R*) substrate analog correctly but showed a strong preference for the unnatural (2*S*) stereoisomer, reducing it mostly in the correct stereochemical sense. DEBS KR2 reduced a high proportion of this unnatural substrate in the wrong stereochemical sense.

methyl-bearing carbon atom [C-2 of the original (2*S*)-methylmalonyl extender] is epimerized at some stage after acylation of the PKS. Together with inversion during condensation, this epimerization would give (2*S*)-2-methyl-3-ketoacyl-ACP1, in which the methyl group has the stereochemistry seen in the final macrolactone ring [5]. Exactly how the epimerization is achieved is uncertain. It may occur postcondensation on the 2-methyl-3-ketoacyl-ACP. With synthetic 2-methyl-3-ketoacyl-*N*-acetylcysteamine (NAC) thioesters, the diketide C-2 proton is made acidic by the β-ketone and thioester carbonyl groups and exchanges rapidly in D_2O [6]. PKS modules do not contain epimerase domains and it has been suggested that they suppress or favor epimerization by restricting or allowing access of the β-ketoester to water [6]. Epimerization after condensation could mean that both unepimerized (2*R*) and epimerized (2*S*) chains are available to downstream enzymes such as the KR or the KS of the next module, which would then contribute to determining the methyl stereochemistry by selecting only 2*S* stereoisomers for further processing. While available evidence favors this view [7], it is still possible that epimerization is brought about by some other unexpected mechanism. Although β-ketone reduction would most likely prevent further C-2 epimerization, it is not essential for fixing the methyl stereochemistry. DEBS module 3 transfers an epimerized, but unreduced, (2*S*) 2-methyl-3-ketoacyl chain to the KS of module 4.

The KR domains of modular PKSs generate the two types of alcohol stereochemistry by adding hydride ions to opposite faces of β-ketone groups [8, 9]. B-type KRs generate products with the same alcohol stereochemistry as the (3*R*)-3-hydroxyacyl-ACP chains that feature in fatty acid biosynthesis. A-type KRs give the same alcohol stereochemistry as the (3*S*)-3-hydroxyacyl-CoA intermediates in fatty acid breakdown [10]. B-type KRs have a few fairly well-conserved amino acid residues that are absent from A-type KRs, whereas A-type KRs have a conserved tryptophan residue that is absent from B-type KRs [10, 11].

Cane, Khosla, and coworkers reversed the stereochemistry of an alcohol in a polyketide product by replacing the A-type DEBS KR2 with the B-type KR4 from the rapamycin PKS (RAPS) [12]. The transplanted RAPS KR4 still carried out B-type ketoreduction even when faced with a β-ketoester that differed from its normal substrate at C-2 and C-4. Further investigations have yielded more complex results, particularly studies on A-type KRs in epimerizing modules [6, 7, 13]. The interpretation of these findings is complicated by the uncertainties surrounding the epimerization step. In their latest study [1], Leadlay and coworkers make a significant advance by overproducing individual KR domains in an active form. To a large extent, this approach separates the ketoreduction step from the unknowns of C-2 epimerization. Five different KR domains were overproduced. With each of these KRs, a racemic mixture of (2*RS*)-2-methyl–3-ketopentanoyl-NAC thioesters was used to investigate the intrinsic stereoselectivity (ability to discriminate between 2*R* and 2*S* substrates) and stereospecificity (ability to catalyze ketoreduction in a single direction). The results are summarized in Figure 1.

The isolated DEBS KR1 acted only on its natural 2*S* diketide substrate and carried out stereospecific ketoreduction to generate the (2*S*, 3*R*) thioester as sole product. The KR1 from the tylosin PKS also acted almost exclusively on its natural diketide, in this case 2*R*, and carried out stereospecific ketoreduction to give a (2*R*, 3*R*) product. These two B-type KRs were capable of selecting their natural substrates from the racemic mixture and catalyzed ketoreduction in the same sense as their counterparts embedded in intact PKS modules. The intrinsic properties of these isolated domains are consistent with the hypothesis that these KRs can determine the methyl stereochemistry and the alcohol stereochemistry in a 2-methyl-3-hydroxyacyl intermediate [6].

The results with the three A-type KRs were less straightforward. During biosynthesis of 6-deoxyerythronolide B (6-dEB), DEBS KR2, KR5, and KR6 domains reduce 2*R* substrates to (2*R*, 3*S*) products. The isolated DEBS KR2, KR5, and KR6 reduced the analogs of their natural substrates stereospecifically, generating (2*R*, 3*S*) products from the 2*R* substrates. A surprising finding was that with all three of these isolated KRs, most

of the reaction products resulted from reduction of the unnatural 2*S* stereoisomer (Figure 1). DEBS KR5 and KR6 reduced this "incorrect" 2*S* substrate mostly, but not completely, in the normal sense to give the (2*S*, 3*S*) product. DEBS KR2 reduced a significant proportion of the unnatural substrate in the wrong stereochemical sense, to give the (2*S*, 3*R*) diketide as 20% of the total reduced products.

These results seem to suggest that a strong preference for 2*S* substrates is itself an intrinsic property of A-type KRs. During 6-dEB biosynthesis, the DEBS KR2, KR5, and KR6 domains are housed in nonepimerizing modules, and by default would encounter only 2*R* substrates, which they reduce stereospecifically to give (2*R*, 3*S*) products. In an epimerizing module, a preference for (2*S*)-2-methyl 3-ketoesters could assist the KR in selecting epimerized rather than unepimerized substrates, and to produce (2*S*, 3*S*)-2-methyl-3-hydroxyacyl intermediates. Further work will be required to ascertain whether DEBS KR2, KR5, and KR6 show different stereoselectivity and more rigorous stereospecificity given longer chain ketoacyl thioesters that more closely resemble their natural substrates.

The tendency of the isolated A-type KRs to give mostly (2*S*, 3*S*) products was not revealed by previous studies where these enzymes were constrained within multienzyme polypeptides of unimodular and bimodular DEBS PKSs [7, 13]. It is possible that in intact modules, the ACP-KR interaction could influence the stereochemical outcome of A-type ketoreduction. With bimodular systems, DEBS KS domains may not accept an (2*S*, 3*S*) *anti* diketide generated by a previous module [14], so that relatively minor products are preferentially incorporated into triketide lactones [7].

The results with isolated KR domains may also be useful in investigating C-2 epimerization. This latest work [1] adds to the evidence [6] that in the first cycle of 6-dEB biosynthesis, DEBS KR1-catalyzed ketoreduction cannot happen without prior C-2 epimerization. Specific inactivation of the epimerase should then abolish KR1 activity against β-ketoesters synthesized by KS1 and ACP1, but not against synthetic substrates such as decalones or (2*S*)-2-methyl-3-ketoacyl thioesters. Regions of module 1 that are essential for C-2 epimerization might then be identified by mutagenesis, or by exchanging stretches of sequence with equivalent regions from a nonepimerizing module. This work could be attempted with the KR2-deleted derivative of DEBS1-TE [6] or the moderately active diketide synthase based on DEBS module 1 [15]. With their work on isolated KR domains, Siskos et al. provide a new avenue for dissecting the stereochemical mechanisms involved in polyketide biosynthesis.

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